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(54) Title: A NOVEL METHOD OF DIAGNOSING, MONITORING, AND STAGING PROSTATE CANCER


(57) Abstract

The present invention provides a new method for detecting, diagnosing, monitoring, staging and prognosticating prostate cancer.


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# A NOVEL METHOD OF DIAGNOSING, MONITORING, AND STAGING PROSTATE CANCER

## FIELD OF THE INVENTION

This invention relates, in part, to newly developed assays for detecting, diagnosing, monitoring, staging, and prognosticating cancers, particularly prostate cancer.

## BACKGROUND OF THE INVENTION

Cancer of the prostate is the most prevalent malignancy in adult males, excluding skin cancer, and is an increasingly prevalent health problem in the United States. In 1996, it was estimated that in the United States, 41,400 deaths would result from this disease, indicating that prostate cancer is second only to lung cancer as the most common cause of death in the same population. If diagnosed and treated early, when the cancer is still confined to the prostate, the chance of cure is significantly higher.

Treatment decisions for an individual are linked to the stage of prostate cancer present in that individual. A common classification of the spread of prostate cancer was developed by the American Urological Association (AUA). The AUA classification divides prostate tumors into four stages, A to D. Stage A, microscopic cancer within prostate, is further subdivided into stages A1 and A2. Sub-stage A1 is a well-differentiated cancer confined to one site within the prostate. Treatment is generally observation, radical prostatectomy, or radiation. Sub-stage A2 is a moderately to poorly differentiated cancer at multiple sites within the prostate. Treatment is radical prostatectomy or radiation. Stage B, palpable lump within the prostate, is further subdivided into stages B1 and B2. In sub-stage B1, the cancer forms a small nodule in one lobe of the prostate. In sub-stage B2, the cancer forms large or multiple nodules, or occurs in both lobes of the prostate. Treatment for both sub-stages B1 and B2 is either radical prostatectomy or radiation.

Stage C is a large cancer mass involving most or all of the prostate and is further subdivided into two stages. In sub-stage C1, the cancer forms a continuous mass that may have extended beyond the prostate. In sub-stage C2, the cancer forms a continuous mass that invades the surrounding tissue. Treatment for both these sub-stages is radiation with or without drugs. The fourth stage is metastatic cancer and is also subdivided into two stages. In sub-stage D1, the cancer appears in the lymph nodes of the pelvis. In sub-stage D2, the cancer involves tissues beyond lymph nodes. Treatment for both these sub-stages is systemic drugs to address the cancer as well as pain.

However, current prostate cancer staging methods are limited. As many as 50% of prostate cancers initially staged as A2, B, or C are actually stage D, metastatic. Discovery of metastasis is significant because patients with metastatic cancers have a poorer prognosis and require significantly different therapy than those with localized cancers. The five year survival rates for patients with localized and metastatic prostate cancers are 93% and 29%, respectively.

Accordingly, there is a great need for increasingly sensitive methods for the staging of a cancer in a human to determine whether or not such cancer has metastasized and for monitoring the progress of a cancer in a human.

In the present invention, methods are provided for detecting, diagnosing, monitoring, staging and prognosticating cancers, particularly prostate cancer via seven (7) Prostate Specific Genes (PSG). The seven PSGs refer, among other things, to native proteins expressed by the genes comprising the polynucleotide sequences of any of SEQ ID NO: 1, 2, 3, 4, 5, 6 or 7. In the alternative, what is meant by the seven PSGs as used herein, means the native mRNAs encoded by the genes comprising any of the polynucleotide sequences of SEQ ID NO: 1, 2, 3, 4, 5, 6 or 7 or levels of the genes comprising

any of the polynucleotide sequences of SEQ ID NO: 1, 2, 3, 4, 5, 6 or 7.

Other objects, features, advantages and aspects of the present invention will become apparent to those of skill in the art from the following description. It should be understood, however, that the following description and the specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only. Various changes and modifications within the spirit and scope of the disclosed invention will become readily apparent to those skilled in the art from reading the following description and from reading the other parts of the present disclosure.

**SUMMARY OF THE INVENTION**

Toward these ends, and others, it is an object of the present invention to provide a method for diagnosing the presence of prostate cancer in a patient which comprises measuring levels of PSG in a sample of cells, tissue or bodily fluid from the patient and comparing the measured levels of PSG with levels of PSG in preferably the same cells, tissue, or bodily fluid type of a control, wherein an increase in the measured PSG levels in the patient versus levels of PSG in the control is associated with prostate cancer.

Another object of the present invention is to provide a method of diagnosing metastatic prostate cancer in a patient which comprises measuring PSG levels in a sample of cells, tissue, or bodily fluid from the patient and comparing the measured PSG levels with levels of PSG in preferably the same cells, tissue, or bodily fluid type of a control, wherein an increase in measured PSG levels in the patient versus levels of PSG in the control is associated with a cancer which has metastasized.

Another object of the present invention is to provide a method of staging prostate cancer in a patient which comprises identifying a patient having prostate cancer,

measuring levels of PSG in a sample of cells, tissues, or bodily fluid obtained from the patient, and comparing the measured PSG levels with levels of PSG in preferably the same cells, tissue or bodily fluid type of a control. An increase in measured PSG levels in the patient versus PSG levels in the control can be associated with a cancer which is progressing while a decrease or equivalent level of PSG measured in the patient versus the control can be associated with a cancer which is regressing or in remission.

Another object of the present invention is to provide a method of monitoring prostate cancer in a patient for the onset of metastasis. The method comprises identifying a patient having prostate cancer that is not known to have metastasized, periodically measuring levels of PSG in a sample of cells, tissues, or bodily fluid obtained from the patient, and comparing the measured PSG levels with levels of PSG in preferably the same cells, tissue, or bodily fluid type of a control, wherein an increase in measured PSG levels versus control PSG levels is associated with a cancer which has metastasized.

Yet another object of the present invention is to provide a method of monitoring the change in stage of prostate cancer in a patient which comprises identifying a patient having prostate cancer, periodically measuring levels of PSG in a sample of cells, tissue, or bodily fluid obtained from the patient, and comparing the measured PSG levels with levels of PSG in preferably the same cells, tissues, or bodily fluid type of a control wherein an increase in measured PSG levels versus the control PSG levels is associated with a cancer which is progressing and a decrease in the measured PSG levels versus the control PSG levels is associated with a cancer which is regressing or in remission.

Other objects, features, advantages and aspects of the present invention will become apparent to those of skill in the art from the following description. It should be

understood, however, that the following description and the specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only. Various changes and modifications within the spirit and scope of the disclosed invention will become readily apparent to those skilled in the art from reading the following description and from reading the other parts of the present disclosure.

**DESCRIPTION OF THE INVENTION**

The present invention relates to diagnostic assays and methods, both quantitative and qualitative for detecting, diagnosing, monitoring, staging, and prognosticating cancers by comparing levels of PSG measured in a patient with levels of PSG in a control. What is meant by "levels of PSG" as used herein, means levels of the native protein expressed by the gene comprising the polynucleotide sequence of any of SEQ ID NO: 1, 2, 3, 4, 5, 6 or 7. In the alternative, what is meant by "levels of PSG" as used herein, is levels of the native mRNA encoded by the gene comprising any of the polynucleotide sequence of SEQ ID NO: 1, 2, 3, 4, 5, 6 or 7 or levels of the gene comprising any of the polynucleotide sequence of SEQ ID NO: 1, 2, 3, 4, 5, 6 or 7. Such levels are preferably measured in at least one of cells, tissues and/or bodily fluids, and includes determination of both normal and abnormal levels of PSGs. Thus, for instance, a diagnostic assay in accordance with the invention for diagnosing overexpression of PSG protein compared to control bodily fluids, cells, or tissue samples may be used to diagnose the presence of cancers, including prostate cancer. Any of the seven PSGs may be measured alone in the methods of the invention, all together or in various combinations of the seven PSGs.

By "control" it is meant a human patient without cancer and/or non cancerous samples from the patient, also referred to herein as a normal human control; in the methods for diagnosing or monitoring for metastasis, control may also

include samples from a human patient that is determined by reliable methods to have prostate cancer which has not metastasized.

All the methods of the present invention may optionally include measuring the levels of other cancer markers as well as PSG. Other cancer markers, in addition to PSG, useful in the present invention will depend on the cancer being tested and are known to those of skill in the art. For example, simultaneous testing for increases in PSA as well as increases in PSG are also within the scope of the present invention and believed to provide a higher level of assurance that such cancer being tested is metastatic or the onset of metastasis has occurred.

***Diagnostic Assays***

The present invention provides methods for diagnosing the presence of prostate cancer by analyzing for changes in levels of PSG in cells, tissues or bodily fluids compared with levels of PSG in cells, tissues or bodily fluids of preferably the same type from a normal human control, wherein an increase in levels of PSG in the patient versus the normal human control is associated with the presence of prostate cancer. Without limiting the instant invention, typically, for a quantitative diagnostic assay a positive result indicating the patient being tested has cancer is one in which cells, tissues, or bodily fluid levels of the cancer marker, such as PSG, are at least two times higher, and most preferably are at least five times higher, than in preferably the same cells, tissues, or bodily fluid of a normal human control.

The present invention also provides a method of diagnosing metastatic prostate cancer in a patient having prostate cancer which has not yet metastasized for the onset of metastasis. In the method of the present invention, a human cancer patient suspected of having prostate cancer which may have metastasized (but which was not previously known to have metastasized) is identified. This is accomplished by a

variety of means known to those of skill in the art. For example, in the case of prostate cancer, patients are typically diagnosed with prostate cancer following traditional detection methods.

In the present invention, determining the presence of PSG in cells, tissues, or bodily fluid, is particularly useful for discriminating between prostate cancer which has not metastasized and prostate cancer which has metastasized.

Existing techniques have difficulty discriminating between prostate cancer which has metastasized and prostate cancer which has not metastasized and proper treatment selection is often dependent upon such knowledge.

In the present invention, the cancer marker levels measured in such cells, tissue, or bodily fluid are PSGs, and are compared with levels of PSG in preferably the same cells, tissue, or bodily fluid type of a normal human control. That is, if the cancer marker being observed is just PSG in serum, this level is preferably compared with the level of PSG in serum of a normal human patient. An increase in the PSG in the patient versus the normal human control is associated with prostate cancer which has metastasized.

Without limiting the instant invention, typically, for a quantitative diagnostic assay a positive result indicating the cancer in the patient being tested or monitored has metastasized is one in which cells, tissues, or bodily fluid levels of the cancer marker, such as PSG, are at least two times higher, and most preferable are at least five times higher, than in preferably the same cells, tissues, or bodily fluid of a normal patient.

***Staging***

The invention also provides a method of staging prostate cancer in a human patient.

The method comprises identifying a human patient having such cancer and analyzing a sample of cells, tissues, or bodily fluid from such patient for PSG. Then, the method

compares PSG levels in such cells, tissues, or bodily fluid with levels of PSG in preferably the same cells, tissues, or bodily fluid type of a normal human control sample, wherein an increase in PSG levels in the patient versus the normal human control is associated with a cancer which is progressing and a decrease in the levels of PSG is associated with a cancer which is regressing or in remission.

***Monitoring***

Further provided is a method of monitoring prostate cancer in a human having such cancer for the onset of metastasis. The method comprises identifying a human patient having such cancer that is not known to have metastasized; periodically analyzing a sample of cells, tissues, or bodily fluid from such patient for PSG; and comparing the PSG levels in such cells, tissue, or bodily fluid with levels of PSG in preferably the same cells, tissues, or bodily fluid type of a normal human control sample, wherein an increase in PSG levels in the patient versus the normal human control is associated with a cancer which has metastasized.

Further provided by this invention is a method of monitoring the change in stage of prostate cancer in a human having such cancer. The method comprises identifying a human patient having such cancer; periodically analyzing a sample of cells, tissue, or bodily fluid from such patient for PSG; comparing the PSG levels in such cells, tissue, or bodily fluid with levels of PSG in preferably the same patient.

Monitoring such patient for onset of metastasis is periodic and preferably done on a quarterly basis. However, this may be more or less frequent depending on the cancer, the particular patient, and the stage of the cancer.

***Assay Techniques***

Assay techniques that can be used to determine levels of gene expression, such as PSG of the present invention, in a sample derived from a host are well-known to those of skill in the art. Such assay methods include radioimmunoassays,

reverse transcriptase PCR (RT-PCR) assays, immunohistochemistry assays, *in situ* hybridization assays, competitive-binding assays, Western Blot analyses and ELISA assays. Among these, ELISAs are frequently preferred to diagnose a gene's expressed protein in biological fluids. An ELISA assay initially comprises preparing an antibody, if not readily available from a commercial source, specific to PSG, preferably a monoclonal antibody. In addition a reporter antibody generally is prepared which binds specifically to PSG. The reporter antibody is attached to a detectable reagent such as radioactive, fluorescent or enzymatic reagent, for example horseradish peroxidase enzyme or alkaline phosphatase.

To carry out the ELISA, antibody specific to PSG is incubated on a solid support, e.g., a polystyrene dish, that binds the antibody. Any free protein binding sites on the dish are then covered by incubating with a non-specific protein such as bovine serum albumin. Next, the sample to be analyzed is incubated in the dish, during which time PSG binds to the specific antibody attached to the polystyrene dish. Unbound sample is washed out with buffer. A reporter antibody specifically directed to PSG and linked to horseradish peroxidase is placed in the dish resulting in binding of the reporter antibody to any monoclonal antibody bound to PSG. Unattached reporter antibody is then washed out. Reagents for peroxidase activity, including a colorimetric substrate are then added to the dish. Immobilized peroxidase, linked to PSG antibodies, produces a colored reaction product. The amount of color developed in a given time period is proportional to the amount of PSG protein present in the sample. Quantitative results typically are obtained by reference to a standard curve.

A competition assay may be employed wherein antibodies specific to PSG attached to a solid support and labeled PSG and a sample derived from the host are passed over

the solid support and the amount of label detected attached to the solid support can be correlated to a quantity of PSG in the sample.

Nucleic acid methods may be used to detect PSG mRNA as a marker for prostate cancer. Polymerase chain reaction (PCR) and other nucleic acid methods, such as ligase chain reaction (LCR) and nucleic acid sequence based amplification (NASABA), can be used to detect malignant cells for diagnosis and monitoring of various malignancies. For example, reverse-transcriptase PCR (RT-PCR) is a powerful technique which can be used to detect the presence of a specific mRNA population in a complex mixture of thousands of other mRNA species. In RT-PCR, an mRNA species is first reverse transcribed to complementary DNA (cDNA) with use of the enzyme reverse transcriptase; the cDNA is then amplified as in a standard PCR reaction. RT-PCR can thus reveal by amplification the presence of a single species of mRNA. Accordingly, if the mRNA is highly specific for the cell that produces it, RT-PCR can be used to identify the presence of a specific type of cell.

Hybridization to clones or oligonucleotides arrayed on a solid support (i.e., gridding) can be used to both detect the expression of and quantitate the level of expression of that gene. In this approach, a cDNA encoding the PSG gene is fixed to a substrate. The substrate may be of any suitable type including but not limited to glass, nitrocellulose, nylon or plastic. At least a portion of the DNA encoding the PSG gene is attached to the substrate and then incubated with the analyte, which may be RNA or a complementary DNA (cDNA) copy of the RNA, isolated from the tissue of interest.

Hybridization between the substrate bound DNA and the analyte can be detected and quantitated by several means including but not limited to radioactive labeling or fluorescence labeling of the analyte or a secondary molecule designed to detect the hybrid. Quantitation of the level of

gene expression can be done by comparison of the intensity of the signal from the analyte compared with that determined from known standards. The standards can be obtained by *in vitro* transcription of the target gene, quantitating the yield, and then using that material to generate a standard curve.

The above tests can be carried out on samples derived from a variety of patients' cells, bodily fluids and/or tissue extracts (homogenates or solubilized tissue) such as from tissue biopsy and autopsy material. Bodily fluids useful in the present invention include blood, urine, saliva, or any other bodily secretion or derivative thereof. Blood can include whole blood, plasma, serum, or any derivative of blood.

**EXAMPLES**

The present invention is further described by the following examples. These examples are provided solely to illustrate the invention by reference to specific embodiments. These exemplifications, while illustrating certain specific aspects of the invention, do not portray the limitations or circumscribe the scope of the disclosed invention.

**EXAMPLE 1: PSGs**

Searches were carried out and PSGs identified using the following Search Tools as part of the LIFESEQ® database available from Incyte Pharmaceuticals, Palo Alto, CA:

1. Library Comparison (compares one library to one other library) allows the identification of clones expressed in tumor and absent or expressed at a lower level in normal tissue.

2. Subsetting is similar to library comparison but allows the identification of clones expressed in a pool of libraries and absent or expressed at a lower level in a second pool of libraries.

3. Transcript Imaging lists all of the clones in a single library or a pool of libraries based on abundance. Individual clones can then be examined using Electronic Northerns to determine the tissue sources of their component ESTs.

4. Protein Function: Incyte has identified subsets of ESTs with a potential protein function based on homologies to known proteins. Some examples in this database include Transcription Factors and Proteases. Some leads were identified by searching in this database for clones whose component ESTs showed disease specificity.

Electronic subtractions, transcript imaging and protein function searches were used to identify clones, whose component ESTs were exclusively or more frequently found in libraries from specific tumors. Individual candidate clones were examined in detail by checking where each EST originated.

**Table 1:**

| SEQ ID NO: | Clone ID # | Gene ID # | |
|---|---|---|---|
| 1 | 1550426 | 244673 | Protein Function (Transcription Factors) |
| 2 | 1255804 | 14878 | Subsetting |
| 3 | 1808432 | 255819 | Subsetting |
| 4 | 3930803 | none | Subsetting |
| 5 | 645804 | 235032 | Subsetting |
| 6 | 1862352 | 221558 | Subsetting |
| 7 | 1450626 | 236019 | Subsetting |

**EXAMPLE 2: Measurement of SEQ ID NO:1; Clone ID # 1550426; Gene ID #244673 (pro101)**

The example is carried out using standard techniques, which are well known and routine to those of skill in the art,

except where otherwise described in detail. Routine molecular biology techniques of the following example are carried out as described in standard laboratory manuals, such as Sambrook et al., MOLECULAR CLONING: A LABORATORY MANUAL, 2nd Ed.; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989).

***Relative Quantitation of Gene Expression***

Real-time quantitative PCR with fluorescent Taqman probes is a quantitative detection system utilizing the 5'-3' nuclease activity of Taq DNA polymerase. The method uses an internal fluorescent oligonucleotide probe (Taqman) labeled with a 5' reporter dye and a downstream, 3' quencher dye. During PCR, the 5'-3' nuclease activity of Taq DNA polymerase releases the reporter, whose fluorescence can then be detected by the laser detector of the Model 7700 Sequence Detection System (PE Applied Biosystems, Foster City, CA, USA).

Amplification of an endogenous control is used to standardize the amount of sample RNA added to the reaction and normalize for Reverse Transcriptase (RT) efficiency. Either cyclophilin, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) or 18S ribosomal RNA (rRNA) is used as this endogenous control. To calculate relative quantitation between all the samples studied, the target RNA levels for one sample are used as the basis for comparative results (calibrator). Quantitation relative to the "calibrator" is obtained using the standard curve method or the comparative method (User Bulletin #2: ABI PRISM 7700 Sequence Detection System).

To evaluate the tissue distribution, and the level of pro101 (SEQ ID NO:1) in normal and tumor tissue, total RNA was extracted from tumor and matched normal adjacent tissues and from unmatched tumor and normal tissues. Subsequently, first strand cDNA was prepared with reverse transcriptase and the polymerase chain reaction carried out using primers and Taqman probe specific to pro101 (SEQ ID NO:1). The results

were obtained using the ABI PRISM 7700 Sequence Detector. The absolute numbers are relative levels of expression of pro101 (SEQ ID NO:1) compared to the calibrator.

The absolute numbers are depicted in the following Table 2 as relative levels of expression in 12 normal tissues of pro101 (SEQ ID NO:1) compared to kidney (calibrator). These RNA samples were generated by pooling samples from a particular tissue from different individuals.

**Table 2: Relative levels of pro101 Expression in Pooled Samples**

| Tissue | NORMAL |
|---|---|
| Brain | 1.2 |
| Heart | 2 |
| Kidney | 1 |
| Liver | 7.2 |
| Lung | 48.2 |
| Mammary | 2.5 |
| Prostate | 1418.4 |
| Spleen | 1.6 |
| Small | 1.9 |
| Testis | 57.3 |
| Thymus | 1.3 |
| Uterus | 7.6 |

The relative levels of expression in Table 2 show that for the PSG pro101 (SEQ ID NO:1) mRNA expression is more than 20 fold higher in the pool of normal prostate compared with the other 11 normal tissue pools analyzed. These results demonstrate that mRNA expression of the PSG is highly specific for prostate.

The tissues shown in Table 2 correspond to pools of samples from different individuals. The tissues shown in the following Table 3 were obtained from individuals and are not pooled. Hence the values for mRNA expression levels shown in Table 2 cannot be directly compared to the values shown in Table 3.

The absolute numbers in Table 3 are relative levels of expression of pro101 (SEQ ID NO:1) compared to kidney (calibrator), in 60 pairs of matching samples. Each matching pair contains the cancer sample for a particular tissue and the normal adjacent sample for that same tissue from the same individual. The results from 3 unmatched ovary tumor, 3 unmatched normal ovary, 1 unmatched mammary tumor and 1 unmatched normal mammary gland are also shown.

**Table 3: Relative Levels of pro101 Expression in Individual Samples**

| TISSUE | CANCER | MATCHING | UNMATCHED |
|---|---|---|---|
| Prostate 1 | 103.9 | 0 | |
| Prostate 2 | 2219 | 84.2 | |
| Prostate 3 | 5048.2 | 3623.6 | |
| Prostate 4 | 11052.3 | 2029.4 | |
| Prostate 5 | 229.1 | 41.1 | |
| Prostate 6 | 57.9 | 25.3 | |
| Prostate 7 | 58.5 | 57.069 | |
| Prostate 8 | 1074.6 | 610.8 | |
| Prostate 9 | 32.7 | 79.3 | |
| Prostate 10 | 15.8 | 2.09 | |
| Prostate 11 | 436.4 | 438 | |
| Prostate 12 | 49.5 | 59.3 | |
| Prostate 13 | 128 | 56 | |
| Bladder 1 | 0 | 0 | |
| Bladder 2 | 0 | 0 | |
| Bladder 3 | 0.7 | 0 | |
| Colon 1 | 0 | 0 | |
| Colon 2 | 0 | 0 | |
| Colon 3 | 0 | 0 | |
| Colon 4 | 3.3 | 1.9 | |
| Colon 5 | 0.1 | 0.8 | |
| Colon 6 | 0 | 0 | |
| Lung 1 | 0 | 0 | |
| Lung 2 | 0.5 | 1.6 | |
| Lung 3 | 1.4 | 2.1 | |
| Lung 4 | 0 | 0 | |
| Lung 5 | 0 | 0 | |
| Kidney 1 | 0 | 0 | |
| Kidney 2 | 0 | 0 | |
| Kidney 3 | 0 | 0 | |
| Kidney 4 | 0 | 0 | |
| Liver 1 | 1.5 | 5.7 | |
| Liver 2 | 26.9 | 7.9 | |
| Liver 3 | 0 | 0 | |

| | | | |
|---|---|---|---|
| Pancreas 1 | 0.9 | 0.9 | |
| Pancreas 2 | 3 | 0 | |
| Pancreas 3 | 0 | 0 | |
| Pancreas 4 | 0 | 0 | |
| Pancreas 5 | 0 | 0 | |
| Stomach 1 | 0 | 0 | |
| Stomach 2 | 0 | 0 | |
| Stomach 3 | 0 | 0 | |
| Stomach 4 | 0 | 0 | |
| Stomach 5 | 0 | 0 | |
| Sm Int 1 | 0 | 0 | |
| Sm Int 2 | 0 | 0 | |
| Testis 1 | 0 | 0 | |
| Mammary 1 | 4 | 0 | |
| Mammary 2 | 5.6 | 0 | |
| Mammary 3 | 0.5 | 0 | |
| Mammary 4 | 0.4 | 0 | |
| Mammary 5 | 0.5 | | |
| Mammary 6 | | | 0 |
| Endo 1 | 1.6 | 7.6 | |
| Endo 2 | 0 | 0 | |
| Endo 3 | 0 | 0 | |
| Endo 4 | 0.3 | 0.2 | |
| Endo 5 | 5.8 | 5 | |
| Uterus 1 | 0 | 0 | |
| Uterus 2 | 0 | 0 | |
| Uterus 3 | 0 | 0 | |
| Uterus 4 | 2.2 | 2.6 | |
| Ovary 1 | 1.4 | | |
| Ovary 2 | | | 11.6 |
| Ovary 3 | 1.5 | | |
| Ovary 4 | | | 22.9 |
| Ovary 5 | 0 | | |
| Ovary 6 | | | 1.8 |

Among 128 samples in Table 3 representing 14 different tissues, the higher levels of expression are consistently in prostate tissues. These results confirm the tissue specificity results obtained with normal samples shown in Table 2. Table 2 and Table 3 represent a combined total of 140 samples in 18 human tissue types. Sixty-eight samples representing 13 different tissue types excluding prostate had no detected pro101 mRNA (Table 3). In 4 tissues (stomach small intestine kidney and testis) no pro101 (SEQ ID NO:1) mRNA was detected for any sample tested from individuals (Table 3). Expression of this PSG was detected in testis in the pooled normal sample (Table 3). The median expression in

prostate cancer samples in Table 3 is 166.5 units. Excluding Ovary 4 (Normal), only 1 sample in Table 3, Liver 2 (Cancer), is greater than 10% of this value.

Comparisons of the level of mRNA expression in prostate tumor samples and the normal adjacent tissue from the same individuals are also shown in Table 3. The PSG pro101 (SEQ ID NO:1) is expressed at higher levels in 9 of 13 (69%) prostate cancer tissues (Prostate 1, 2, 3, 4, 5, 6, 8, 10 and 13) compared with the corresponding normal adjacent tissue. The level of expression of this PSG is lower in prostate tumor compared to normal adjacent tissue in two samples (Prostate 9 and 12). Equivalent levels of expression were detected in two matched samples (Prostate 7 and 11). Previous mRNA expression analysis for genes coding for the diagnostic markers PSA and PLA2 showed higher expression of the mRNA in 40% to 80% of the tumor samples compared to matching normal adjacent tissue. Higher expression in the tumor sample compared to the corresponding normal adjacent tissue is observed for Bladder 3, Colon 4, Liver 2, Pancreas 2, Endometrium 5 and. Mammary 1, 2 and 3. Higher expression in the normal adjacent samples is observed for Colon 5, Lung 2, Lung 3, Liver 1, Endometrium 1 and Uterus 4. However, the levels detected are in most cases comparable amongst the different tissues and low compared to levels found in most prostate tissues.

The high level of tissue specificity, plus the mRNA overexpression in 9 of 13 of the prostate tumor samples tested compared to the normal adjacent tissues are believed to make the PSG, pro101 (SEQ ID NO:1) a good diagnostic marker for detection of prostate cancer using mRNA.

**What is Claimed is:**

1. A method for diagnosing the presence of prostate cancer in a patient comprising:

(a) measuring levels of PSG in a sample of cells, tissue or bodily fluid obtained from the patient; and

(b) comparing the measured levels of PSG with levels of PSG in a sample of cells, tissue or bodily fluid obtained from a control, wherein an increase in measured levels of PSG in the patient versus the PSG levels in the control is associated with the presence of prostate cancer.

2. A method of diagnosing metastatic prostate cancer in a patient comprising:

(a) measuring levels of PSG in a sample of cells, tissue, or bodily fluid obtained from the patient; and

(b) comparing the measured levels of PSG with levels of PSG in a sample of cells, tissue, or bodily fluid obtained from a control, wherein an increase in measured PSG levels in the patient versus the PSG levels in the control is associated with a cancer which has metastasized.

3. A method of staging prostate cancer in a patient comprising:

(a) identifying a patient suffering from prostate cancer;

(b) measuring levels of PSG in a sample of cells, tissue, or bodily fluid obtained from the patient; and

(c) comparing the measured levels of PSG with levels of PSG in a sample of cells, tissue, or bodily fluid obtained from a control, wherein an increase in the measured levels of PSG versus the levels of PSG in the control is associated with a cancer which is progressing and a decrease in the measured levels of PSG versus the levels of PSG in the control is associated with a cancer which is regressing or in remission.

4. A method of monitoring prostate cancer in a patient for the onset of metastasis comprising:

(a) identifying a patient having prostate cancer that is not known to have metastasized;

(b) periodically measuring PSG levels in samples of cells, tissue, or bodily fluid obtained from the patient; and

(c) comparing the periodically measured levels of PSG with levels of PSG in cells, tissue, or bodily fluid obtained from a control, wherein an increase in any one of the periodically measured levels of PSG in the patient versus the levels of PSG in the control is associated with a cancer which has metastasized.

5. A method of monitoring changes in a stage of prostate cancer in a patient comprising:

(a) identifying a patient having prostate cancer;

(b) periodically measuring levels of PSG in samples of cells, tissue, or bodily fluid obtained from the patient; and

(c) comparing the measured levels of PSG with levels of PSG in a sample of the same cells, tissue, or bodily fluid of a control, wherein an increase in any one of the periodically measured levels of PSG versus levels of PSG in the control is associated with a cancer which is progressing in stage and a decrease in any one of the periodically measured levels of PSG versus the levels of PSG in the control is associated with a cancer which is regressing in stage or in remission.

6. The method of claim 1, 2, 3, 4 or 5 wherein the PSG comprises SEQ ID NO:1.

SEQUENCE LISTING

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Sun, Yangming
Cafferkey, Robert

<120> A Novel Method of Diagnosing, Monitoring and Staging Prostate Cancer

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ccagcagcag cgccagcagc agcgccccgc gcgcgcccat ggcctcctct cccgcggtg  1079
```

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/10548

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(6) : C12Q 1/68; G01N 33/574
US CL : 435/6, 7.23

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6, 7.23

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

DIALOG, APS

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | US 5,506,106 A (CROCE et al.) 09 April 1996, col. 1, lines 50-65. | 1-5 |
| X | DEGUCHI, T. et al. Detection of Micrometastatic Prostate Cancer Cells in the Bone Marrow of Patients with Prostate Cancer. British Journal of Cancer. 1997, Vol. 75, No. 5, pages 634-638, especially page 634. | 1-5 |
| P,Y | AN, G. et al. Cloning of Prostate-Specific Genes that are Suppressed in Metastatic Prostate Cancer by a PCR Southen Differential Hybridization Method. Cell and Tumor Biology. March 1998, Vol. 39, page 208, especially page 208. | 1-6 |

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

| | | | |
|---|---|---|---|
| * | Special categories of cited documents: | "T" | later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention |
| "A" | document defining the general state of the art which is not considered to be of particular relevance | "X" | document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone |
| "E" | earlier document published on or after the international filing date | "Y" | document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art |
| "L" | document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) | "&" | document member of the same patent family |
| "O" | document referring to an oral disclosure, use, exhibition or other means | | |
| "P" | document published prior to the international filing date but later than the priority date claimed | | |

| | |
|---|---|
| Date of the actual completion of the international search<br>12 AUGUST 1999 | Date of mailing of the international search report<br>09 SEP 1999 |
| Name and mailing address of the ISA/US<br>Commissioner of Patents and Trademarks<br>Box PCT<br>Washington, D.C. 20231<br>Facsimile No. (703) 305-3230 | Authorized officer<br>D. Lawrence for<br>YVONNE EYLER<br>Telephone No. (703) 308-0196 |

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